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I Siah Substrate!

Proteins are targeted to the E3 RING ubiquitin ligase Siah through a PxAxVxP degron motif. In this issue of *Structure*, House et al. (2006) present the structural basis by which Siah recognizes its degron with high affinity and specificity.

The SINA/Siah family of E3 ubiquitin ligases is implicated in the targeted degradation of a diverse range of proteins including β-catenin and the transcriptional repressor Tramtrack (TTK88) (Boulton et al., 2000; Li et al., 1997; Liu et al., 2001; Matsuzawa and Reed, 2001; Tang et al., 1997). The majority of the known SINA/Siah binding proteins possess the heptad sequence, Pro-X-Ala-X-Val-X-Pro (where X is not conserved). Since Siah interaction with proteins bearing this motif leads many to be ubiquitinated, the motif has been designated the Siah degron (House et al., 2003). Aside from substrates such as TIEG1, which interacts directly with Siah (Johnsen et al., 2002), the degron motif can also facilitate the interaction of Siah with adaptor proteins such as SIP (*S*iah-*i*nteracting *p*rotein) and Phyllopod (PHYL), which in turn interact with other proteins to render them substrates of Siah.

Drosophila SINA (seven *in a*bsentia) and mammalian Siah (seven *in a*bsentia *h*omologue) possess a domain architecture consisting of an E2 binding RING domain, two zinc finger motifs, and a substrate/adaptor binding domain (SBD). A previous X-ray crystallographic analysis of the Siah SBD revealed an eight-stranded β sandwich fold bearing close resemblance to the TRAF domain, characteristic of TNF-receptor associated factor (TRAF) proteins (Polekhina et al., 2002). While the SBD and TRAF proteins share a common fold, the TRAF domain engages its interacting partners through a cleft that is occluded by a unique helical segment (residues 246–255) in the Siah SBD. The presence of this segment in the Siah SBD precludes Siah from engaging adaptor proteins and substrates in the manner employed by TRAFs (Polekhina et al., 2002). As such, the mechanism by which the Siah SBD engages its degron motif remained to be determined.

In this issue of *Structure*, House et al. (2006) present an X-ray crystallographic analysis of the Siah1 SBD in complex with a degron-containing peptide of sequence Leu-Arg-$\underline{Pro}_1$-Val_{X2}-$\underline{Ala}_3$-Met_{X4}-$\underline{Val}_5$-Arg_{X6}-$\underline{Pro}_7$-Thr-Val-Arg from Phyllopod, an adaptor that targets TTK88 to Siah for ubiquitination (Boulton et al., 2000; Li et al., 1997; Tang et al., 1997). In the structure, the Phyllopod peptide engages a hydrophobic cleft on the β-barrel sandwich opposite the surface where TRAF domains engage their binding partners. The degron peptide binds parallel to the outermost β1 strand forming a pseudo extension of the β sheet. The conserved Pro, Ala, Val, and Pro residues of the degron orient into the hydrophobic environment of the cleft. Specific binding of Siah to the degron is achieved through specific binding pockets for the alanine and valine residues in degron positions 3 and 5 that are too small to accommodate bulkier side chains. In addition, the side chain of proline in degron position 7 packs in a coplanar manner with the Siah side chain of Trp178. Mutagenesis of key residues within the binding cleft on Siah was shown to disrupt binding to PHYL in vitro and to stabilize TIEG1 in vivo, thus validating the degron recognition mechanism uncovered in their structure analyses (House et al., 2006). While not tested, these same mutations in the Siah SBD would also be predicted to stabilize TKK88, which requires the adaptor PHYL for targeting and, ultimately, ubiquitination by Siah.

Degron recognition by the SBD of Siah was independently verified in a study by Matsuzawa and colleagues (Santelli et al., 2005). In that study, the structure of Siah1 in complex with a degron-containing peptide of sequence Glu-Lys-$\underline{Pro}_1$-Ala_{X2}-$\underline{Ala}_3$-Val_{X4}-$\underline{Val}_5$-Ala_{X6}-$\underline{Pro}_7$-Ile-Thr derived from the adaptor protein SIP was analyzed using both NMR and crystallographic techniques. The SIP and PHYL peptides engage the Siah SBD in a near identical manner. This was somewhat of a surprise considering that the PHYL degron peptide binds the SBD with an apparent 100-fold higher affinity than the SIP degron peptide (House et al., 2003; Santelli et al., 2005). This discrepancy in affinity was rationalized in part by diversity in peptide residues flanking the core degron heptad and differences among the variable positions within the degron itself. In support of this possibility, the residue at variable position 2 is a valine (Val117) in PHYL versus alanine (Ala61) in SIP, and mutation of this position within the PHYL degron to Ala, was shown to reduce binding in vitro (House et al., 2003). The valine in degron position 2 is observed to form a hydrophobic interaction with Val164 and Leu166 in the Siah SBD. While few residues flanking the core degron heptad were visualized in either of the Siah-peptide complexes due to disorder, it was rationalized that degron-flanking basic residues specific to the PHYL peptide may form favorable electrostatic interactions with an acidic patch on the Siah SBD, which could further account for the enhanced binding properties of the PHYL peptide (House et al., 2006; Santelli et al., 2005).

Clearly, differences in residues outside of the conserved degron positions have a pronounced effect on binding affinity. However, the relevance of large differences in binding affinity on Siah-mediated protein ubiquitination remains to be determined. The paper presented by House et al. (2006) in this issue of *Structure* lends insight into the binding mode of Siah with its degron and provides a solid platform for further analysis into the relationship between SINA/Siah and its corresponding adaptors and substrates.

Douglas J. Briant,[1] Derek F. Ceccarelli,[1] and Frank Sicheri[1,2]
[1] Samuel Lunenfeld Research Institute
Mount Sinai Hospital
Toronto, Ontario, M5G 1X5
Canada

[2]Department of Medical Genetics and Microbiology
University of Toronto
1 King's College Circle
Toronto, Ontario, M5S 1A8
Canada

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A Monotopic Membrane Protein Goes Solo

Carnitine palmitoyltransferases (CPTs) are part of the enzymatic system that imports fatty acids into mitochondria. The crystal structure of rat CPT-2 by Rufer et al. (2006) (this issue of *Structure*) reveals a Y-shaped tunnel for binding the CoA and acyl-carnitine substrates and a hydrophobic insert mediating membrane association.

Mitochondrial fatty acid oxidation requires that fatty acids are imported from the cytosol across the two mitochondrial membranes. This process involves conjugation of the fatty acids to carnitine in a reaction catalyzed by carnitine palmitoyltransferase 1 (CPT-1). Acyl-carnitine is transported by a specific carrier (Palmieri, 2004) into the mitochondrial matrix, where it is converted back to acylCoA through a *trans*-esterification reaction carried out by carnitine palmitoyltransferase 2 (CPT-2). AcylCoA can then be metabolized through the fatty acid β-oxidation pathway.

Alterations in the enzymatic activities of the fatty acid translocation system can have profound effects on energy metabolism. Deficiencies of CPT-1 and CPT-2 caused by genetic defects result in severe disorders that can be lethal, particularly in cases of early onset (Stanley, 2004). On the other hand, it is known that inhibition of the CPT enzymes can be of therapeutic value for treatment of non-insulin-dependent type 2 diabetes, and CPT inhibitors are being tested in clinical trials (Giannessi et al., 2003). The rationale for this approach is that inhibition of CPTs reduces gluconeogenesis and increases insulin-mediated glucose uptake. The problem is that isoform-specific inhibitors are needed in order to decrease toxicity and to maximize therapeutic efficacy.

In this issue of *Structure*, Rufer and coworkers describe the crystal structure of rat CPT-2 in complex with an inhibitor under clinical evaluation (Rufer et al., 2006; Giannessi et al., 2003). RatCPT-2 is a monotopic membrane protein with its hydrophilic domain exposed on the matrix side of the inner mitochondrial membrane. Despite its membrane association, the rat enzyme can be expressed in *Escherichia coli* with remarkably high yields. The detergent used for crystallization was n-octyl-β-D-glucopryranoside, and crystals were obtained only at a certain detergent concentration (1.5-fold the critical micelle concentration), underscoring how crucial the detergent and its concentration are for the crystallization of membrane proteins that have large hydrophilic globular domains. The authors employed analytical ultracentrifugation to study protein oligomerization. In the case of membrane proteins, this technique is not straightforward in that it is necessary to achieve so-called gravitational transparency to compensate for the presence of the detergent in solution (Lustig et al., 2000). However, the methodology can be especially useful for identifying those detergents that make the protein monodisperse and therefore can facilitate crystallization. The analytical ultracentrifugation experiments showed that ratCPT-2 solubilized in n-octyl-β-D-glucopryranoside is monodisperse and monomeric. Consistently, a monomeric state is found also in the crystals.

As expected on the basis of sequence homology, the overall structure of ratCPT-2 exhibits the same two-domain folding topology as carnitine acetyltransferase (Jogl and Tong, 2003). A notable feature is the architecture of the active site, which is formed by a "Y-shaped" tunnel at the domain interface. The two arms of the "Y" differ in their hydrophobic character forming the CoA- (more hydrophilic) and acyl binding (hydrophobic) sites, respectively. Carnitine binding and catalysis of the *trans*-esterification reaction occur at the junction of the two arms. Structure-based sequence alignment confirmed that the rat enzyme is highly homologous to the human protein, and therefore it can serve as a valid model to understand the differences in substrate